

ON THE RELATION OF THE INORGANIC SALTS OF  
BLOOD TO THE AUTOMATIC ACTIVITY OF  
A STRIP OF VENTRICULAR MUSCLE

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A THESIS

PRESENTED TO THE BOARD OF UNIVERSITY STUDIES OF THE JOHNS HOPKINS  
UNIVERSITY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

— BY —

CHARLES WILSON GREENE

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# ON THE RELATION OF THE INORGANIC SALTS OF BLOOD TO THE AUTOMATIC ACTIVITY OF A STRIP OF VENTRICULAR MUSCLE.

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IT is the intention in this paper to investigate more fully the conditions of automatism in the tissue of the heart rather than to discuss the question whether the automatism of the heart resides primarily in the muscular tissue or in the nervous mechanism. Brown-Séquard<sup>1</sup> pointed out more than fifty years ago that the more fundamental question regarding the heart's activity is not which tissue is automatic but why or under what conditions either tissue shows automatism. I emphasize the idea of conditions of automatism with intention, for while one may justify himself in setting forth hypotheses for the explanation of results obtained under the multiple conditions of experimentation, still one must fully recognize the impossibility of reaching a decisive solution of a question which at the present time involves so many necessarily unknown factors.

The influence of the blood on the heart's contraction must necessarily be considered both from the physical and from the chemical point of view. The characteristics of the blood are isotonicity with the living tissue, a certain degree of viscosity, and a complex chemical composition. Since the experiments of Nasse<sup>2</sup> in 1869, on the effects of different strengths of sodium chloride solution on the irritability of the gastrocnemius of the frog, isotonicity with the living tissue has been recognized by all physiologists as a requisite of any artificial solution adapted to sustain the activity of living tissue. In the experiments recorded in this paper, the effort has been made to keep this factor constant by using solutions as nearly isotonic as possible, except in a very few special cases. Certain investigators, notably Heffter,<sup>14</sup> have insisted upon the importance of a degree of viscosity in an artificial circulating fluid. Others have disproved, or at least have thrown grave doubts on the validity of this conclusion;



and until further evidence is brought forth we may safely ignore this property in any investigation on the chemical constitution of the blood in its relation to tissue activity.

As a basis for the preparation of artificial solutions of the salts found in blood chemical analyses of blood or serum as given in the literature of the subject are in many respects incomplete. The most common defect is the lack of information as to the exact forms of the salts present, and the proportions in which they exist as free salts and as salts in combination with organic components. Then, too, such salts as are found only in minute quantities have not been quantitatively determined, although from a physiological point of view they may be of very great importance.

The most abundant salts of blood are those of sodium, potassium, calcium, and magnesium. In experiments up to the present, the action of these salts has been studied mainly upon the heart of the frog. The experiments described in this paper were confined to the terrapin, and were performed exclusively on strips of muscle cut from the apex of the ventricle. The strips were taken either from ventricles filled with blood or from ventricles freed of blood as far as possible by irrigation with normal saline solution. In the latter case the heart was isolated and irrigated through an inflow cannula inserted into the left vena cava and an outflow cannula in one of the aortic branches. The coronary arteries vary much in their origin in the terrapin used, but care was always taken to establish as good a coronary circulation as possible by cutting one of the coronary veins.

From two to four slender strips were prepared from each heart by first cutting off the apical two thirds and then splitting this into the requisite number of pieces. In all, over fifty different hearts were used, giving from two to four strips each, and each strip was submitted to from five to ten distinct changes in the experimental conditions. The effect of each change was observed for periods varying from one to many hours. The average duration of experimentation on a single strip was from forty to fifty hours, although many experiments exceeded seventy-two hours and some reached ninety-six to ninety-eight hours. It will readily be seen that the total time of suspension and the number and character of changes preceding any particular test are most important factors in determining the results of that test, and that the unifying of the results of the entire series of experiments is correspondingly difficult.



## METHODS AND APPARATUS.

In 1880 Gaskell demonstrated that a strip of the apex of the terrapin's ventricle would give automatic rhythmic contractions when suspended in a moist chamber and left to itself. This simple experiment offers a method for the study of rhythmic muscular tissue as such. Although an intricate network of nerve fibres interlaces among the muscle cells of the apex of the ventricle, and, according to Berkley,<sup>18</sup> an occasional isolated nerve cell is found in even the apex in the frog and the mouse, still it can scarcely be supposed that a stimulus that acts to produce rhythmic contractions in the isolated strip of the ventricle acts on the terminal nerve elements to the exclusion of the muscle cells. However this may be, whether the automatism resides in the muscle or nerve, the phenomena associated with the rhythm of an isolated strip of muscle seem to admit of a more definite study than the phenomena associated with the activity of the complexly organized heart itself.

In a preliminary experiment made upon a different species of terrapin from that used in the remainder of the experiments, the apex muscle strip of the terrapin's ventricle suspended in a moist chamber remained alive and gave off rhythmic contractions for three days. This preliminary test fully demonstrated the utility of the method, and I have, therefore, adopted this method for the study of the inorganic constituents of blood in their relation to the activity of cardiac muscle. The following pages will, I hope, fully demonstrate the directness, reliability, and simplicity of the method.

My plan of procedure has been, in brief, to suspend a slender strip of the apex of the ventricle (approximately 2.5 cm. long and weighing 0.2 to 0.3 gram) in a moist chamber, attach it to a recording lever under a definite tension, submit it to an artificial fluid by immersing it in the solution or by moistening it in air, and record the resulting phenomena by the lever and by direct observation. The extraordinarily long life of the muscle under such conditions permits a series of experimental changes affecting each strip. By this method one has the very great advantage of placing several strips from the same heart simultaneously in different solutions. The requirements of this method are very simple, and no intricate apparatus is needed. It is necessary only that the muscle be protected from evaporation, and that it be connected with a delicately poised recording lever. The apparatus I adopted has the

additional advantage of all the desirable mechanical conveniences in adjustment.

The muscle was suspended in a glass tube fourteen centimetres in length and of a diameter of one and a half to three centimetres, according to the experiment. The tube was closed at the bottom by a rubber stopper containing a small glass cannula, so arranged as to permit the filling or the complete emptying of the tube without disturbing the heart strip. One side of the upper or inner end of the cannula was drawn out and bent into a small hook for the attachment of the lower end of the muscle strip. The upper end of the heart tube was either covered with oiled paper, or—in the tube used for air experiments—was constricted into a small neck with funnel-shaped outer end, which when filled with a drop of water offered no resistance to the movement of the thread passing through it, yet at the same time prevented evaporation from the heart tube. Silk ligatures were tied around each end of the muscle strip, and one thread closely looped over the glass hook at the top of the stopper-cannula while the other was carried to a lever above, or small glass S-shaped hooks were caught into the ends of the muscle strip. The hooks are preferable for the comparatively large ventricular strips.

A light straw lever was used in these experiments. The lever was balanced on a steel rod one millimetre in diameter by means of a glass hub about twenty-five millimetres long, the ends of which were constricted so that the weight of the lever rested on a very small bearing surface. This device reduced the friction of the lever on the steel wire support to a minimum. The fulcrum of the lever was placed between the attachment of the muscle and the writing point, converting the downward pull on the lever into an upward stroke on the recording cylinder. The lever carried a five-gram weight near the supporting glass hub so adjusted that the total weight on the muscle was approximately one gram. The axis of the lever was supported above the heart tube by means of rods and corks so arranged that the lever could be readily adjusted in any plane. The horizontal adjustment was found extremely convenient, since by it the recording lever could be taken from the recording surface or returned at any moment without in the least disturbing other pieces of apparatus attached to the same stand.

A slow drum was found best adapted to the slow rate usually given by the terrapin's ventricular strip, especially when the experiment con-

tinued through a series of hours including, perhaps, two or three consecutive nights. The particular drum used for most of these experiments had a circumference of forty-seven centimetres, and made one revolution in eleven hours. When parallel experiments were made, as was always the case except in one or two experiments, two and three heart tubes with levers were attached to the same stand, the levers writing one above the other.

The artificial solutions used in these experiments were in every case made in water distilled in glass. The absolute necessity of this precaution in any series of experiments involving the effects of the inorganic salts of blood on living animal tissue was first demonstrated by Locke,<sup>16</sup> whose work has recently been confirmed by Ringer. These investigators showed that minute traces of certain of the heavy metals, such as copper, dissolved from the copper-receiving tanks so often used for distilled water, may completely obscure the effects of the particular salt experimented with. The water used in my experiments was prepared in part by the method devised by Jones and Mackay,<sup>19</sup> except that potassium bichromate was substituted for permanganate of potassium in the first flask according to a recent suggestion of the authors', and that I used a Jena glass condenser tube instead of the block tin tube used by them. More recently the water used was purified by making ordinary laboratory distilled water slightly alkaline with sodium hydroxide and re-distilling in glass.

The sodium and potassium salts used were purified by repeated recrystallization of "chemically pure" salts. Solutions were made by dissolving a weighed quantity of the dry salt in a measured quantity of distilled water. Stock solutions of one per cent strength were prepared from all salts, except, of course, sodium chloride, and solutions for immediate use were made up from these stocks as needed. In the case of the deliquescent calcium chloride a solution was prepared of approximately the strength required, and the amount of calcium in this solution was quantitatively determined by the usual gravimetric method of precipitation as calcium oxalate and conversion to calcium oxide. From this general solution a one per cent stock was prepared.

In the details of experiments the proportions of the various salts used in any given artificial solution are expressed in terms of percentage.



## SERUM.

Before discussing the relation of solutions of the inorganic blood salts to the action of a ventricular strip, let us first consider the behavior of such a strip immersed in its own serum. If an apex strip cut from the heart of a terrapin, *Chrysemys picta*, is suspended in a heart tube and covered with normal terrapin serum, absolutely no rhythmical contractions are developed. If the preparation is not quickly made and is allowed to evaporate slightly during the process, a few single contractions may occur when the strip is first suspended. The number of such contractions does not exceed ten or fifteen, and they occur during the thirty to sixty minutes following suspension.

In five experiments strips were kept in serum from twenty-one to one hundred hours. After thirty to fifty hours an occasional contraction may occur. The longer a strip is kept in serum the more frequent the contractions become, but they do not on an average exceed one an hour in strips kept for a very long time. One strip, number 47d, left undisturbed for seventy-six hours in serum, gave during that time eighty-two single contractions, seventy-five of these occurring during the last half of the time. Meanwhile bacteria appeared in the serum; this was therefore drawn off and the strip left suspended in air, though still moist with serum. The muscle at once began to contract with full normal contractions and continued to do so for six hours, when the recording drum stopped and further record was lost. Another strip was kept in serum for ninety-six hours, and gave only forty contractions during about one half of the time, the record being lost at intervals during the experiment. At the end of ninety-six hours this strip was quiet in a relaxed state, but when changed to 0.6 per cent sodium chloride solution it immediately gave a series of rhythmical contractions. These contractions were more irregular in rate than is usual in a sodium chloride series, such as will be described later. They were at first 1.2 to 1.4 cm. high, and gradually decreased to zero within an hour and a half. After one hundred hours a bath of Ringer's solution produced a strong increase in tone and fibrillation, showing that the muscle was still alive and irritable.

From these examples it will be seen that *normal serum preserves the heart strip in good condition for contraction for a very long time, but does not supply the necessary conditions for the development of rhythmic contractions of ventricular muscle.*

It is well known that an isolated apex of the frog's ventricle will not give contractions when the heart is filled with its own blood, a fact made use of since its discovery by Bernstein in 1876 to support the view that the contractions of the heart are nervous in origin. It is significant, therefore, in light of what I shall present later, that the ventricular strip agrees in its reaction to serum with the apex of the frog's heart isolated by Bernstein's method.

**Modified serum.**— In 1875 Merunowicz<sup>4</sup> found that blood of the sheep or rabbit is most favorable to the development of good contractions in the frog's heart when diluted with 0.6 per cent sodium chloride solution in the ratio of one part of blood to four parts saline. McGuire<sup>5</sup> in 1878 investigated the relative proportions of rabbit serum and 0.6 per cent saline in reference to their sustaining power on the frog's heart. He found that a mixture of serum and saline in the ratio of one to six does not produce maximal contractions,— that one to two is better, while greater concentration of serum is “unfavorable.” At the present day, following these and similar results obtained by other observers, it is customary to dilute rabbit or other mammalian blood with sodium chloride solution when it is to be used for artificial circulation experiments, without inquiring why the dilution improves the blood in its adaptability to the production of good rhythmic contractions. I have repeated on the apex strip the experiments of McGuire on the frog's heart, and have other experimental evidence which I hope will show to what dilute serum owes its efficiency. It has already been shown that the terrapin's own serum in its normal concentration and composition as well as mammalian serum is inefficient in developing rhythmic contractions in a heart strip.

*Experiment 24, December 11, 1897.* A perfectly fresh heart strip was suspended in fresh serum obtained from another terrapin. During the succeeding forty-one hours, twelve contractions were developed. These contractions varied in amplitude from 0.1 cm. to 1.0 cm., and occurred at very irregular intervals. The smaller contractions were submaximal. Sodium chloride 0.6 per cent was then added at successive intervals during ten minutes. After twelve minutes full, strong contractions began which were at first 1.0 cm. high but increased to 1.2 cm. in two or three minutes, then slowly decreased to 0.8 cm. in the two hours following. The rate was quite irregular in this series and the contractions were in groups of varying rates. Ten minutes after the series began one group showed a rate of twenty-eight per minute. As the height decreased the rate became slower and slower. After two

hours contractions came only at long intervals and so continued for twenty-four hours.

*Experiment number 30 b.* An apex strip was immersed in 0.64 per cent sodium chloride solution until the contractions resulting decreased from 1.15 cm. to 0.02 cm. in height. The saline was then drawn off and normal serum introduced, four hours and five minutes after suspension. The strip immediately exhibited incoördinated contractions, and fibrillation. At the same time the strip shortened in consequence of an increase in "tone." In fifteen minutes the fibrillation disappeared and regular contractions of large amplitude began and the tone passed gradually away. The rate of contraction became slower as the contractions became more nearly normal in height, until in fifty minutes the strip remained quiet in a relaxed condition. The muscle was apparently in a state comparable to that of a fresh strip at rest in a serum bath.

After forty minutes of quiet the serum was diluted with 0.6 per cent sodium chloride solution in the ratio of one part serum to two parts sodium chloride. There was no change for sixty minutes, then contractions 1.7 cm. in height began with a very irregular rate. The contractions gradually decreased in rate and ceased in forty minutes. Six hours and twenty-five minutes from the time the serum was first used the serum-saline was further diluted to the proportion of one part serum to six parts sodium chloride. In ten minutes a perfectly regular series of contractions began, and continued for four hours. The rate during this time slowly decreased from 2 to 1.3 per minute, but was otherwise perfectly regular. The amplitude was exceptionally great, 1.7 cm. Between ten hours twenty-five minutes and sixteen hours thirty-five minutes after the serum was first introduced the strip remained for the most part quiet in diastolic state, only occasionally giving a contraction. That the above heart strip was in good condition all the time was shown by the beautiful series procured when later the strip was transferred to 0.6 per cent solution of sodium chloride.

Attention may here be called to the fact that the serum saline series of regular contractions in the above experiment extends over a time as long as or longer than that of many experiments on the frog's heart noted in literature, and that here, as in most of the experiments in this research, the experiment was continued until the after effects were fully determined. For this particular strip I have a continuous record through successive experimental conditions for seventy-three consecutive hours.

From the above examples it may be noted that automatic rhythmic contractions are developed in the ventricular strip by submitting it directly to a bath of serum diluted with a large amount of physiological saline. Also that beats are developed in a strip that has been



exposed to solutions of sodium chloride, 0.6 per cent, and then surrounded by serum. That is, it may be assumed that beats occur during that early period in the process of diffusion when the salts of the serum diffusing into the muscle mass may be assumed to be very much diluted. It must also be noted here that a strip that is quiet in normal serum may be made to beat with perfect rhythm and complete and normal amplitude, although for a variable time, simply by increasing the percentage of calcium salts in the serum.

Further, serum will revive activity in a heart strip after it has been thrown into a state of strong tone accompanied by fibrillation in consequence of the action of other solutions, such as sodium chloride solution containing calcium chloride, or a Ringer's solution used after sodium chloride. The exact type of recovery depends upon the degree of general exhaustion of the strip, *i. e.*, upon its total activity since suspension, and upon the length of time that the strip has been in fibrillation. When such a fibrillating strip is changed to serum it immediately begins to increase in activity, giving stronger and more rapid fibrillary contractions. After a longer or shorter period depending on the above mentioned conditions the strip suddenly ceases fibrillation, partially relaxes, and then begins contractions that are normal in type but irregular in rate and not frequent. (See Figs. 2 and 3.) The contractions obtained from strips that are in a more nearly exhausted state are small at first and gradually increase in height during several hours. Almost invariably, however, the contractions revived by serum become more and more infrequent, and practically cease after five to fifteen hours. This condition is again comparable to that of a fresh strip immersed in serum, except that fatigued strips, it must be remembered, have been under experimentation for several hours and have already expended a great amount of energy in muscular contractions.

Finally, a muscular strip beating for a long time in a solution of inorganic salts may ultimately gradually decrease the amplitude of its contractions and pass into a state from which it never recovers its original amplitude. When in this state it is only slightly revived by serum. This is a state of true exhaustion which will be discussed in more detail later. If such an exhausted strip be immersed in pure serum it will give a series of feeble contractions. These feeble contractions are often of quite a rapid rate, but in height do not even approximate the contraction given by the strip before exhaustion. They quickly disappear again, while serum used on a partially

exhausted strip revives quite normal contractions that persist a relatively long time. By treating the exhausted strip with the proper combination of inorganic salts a similar slight recovery in rate is produced, but never quite so great as with serum. (See experiment 42.)

Serum will revive a strip from the quiescent state — so-called exhaustion — following exposure to the action of solutions of sodium chloride or sodium chloride plus potassium chloride, such as will be described later; or from a state of tone and fibrillation caused by the use of Ringer's solution containing excess of calcium salts upon a strip exhausted by sodium chloride. In fact, serum will apparently revive a heart strip from almost any unfavorable condition brought about by isotonic solutions of the inorganic salts found in the blood, except possibly that condition of true exhaustion which has been briefly mentioned above and is described more fully in the section on exhaustion.

#### SODIUM, POTASSIUM, AND CALCIUM SALTS.

**Sodium chloride.** — Sodium chloride is the most abundant salt in the blood. Its solution in amounts isotonic with the blood has therefore during nearly thirty years been in constant use as the so-called indifferent or normal physiological saline. So far as is now known, it is the only substance that can be used in an artificial circulation medium to preserve the isotonicity with the tissues, and isotonicity seems to be a necessary factor in all experiments on artificial solutions. I have, therefore, in my experiments taken up first the effects of solutions of sodium chloride on the development and maintenance of the automatic contractions of the apex strip of the terrapin's heart.

It was shown long ago by Merunowicz,<sup>4</sup> 1875, that the isolated apex of the frog's ventricle would, after a certain latent period, beat automatically and rhythmically when the heart was filled with 0.6 per cent sodium chloride solution instead of with normal blood or with sheep's blood diluted with 0.6 per cent sodium chloride. Later Aubert,<sup>8</sup> 1881, showed that not only would the isolated apex beat when filled with sodium chloride but that it would again become quiet if the saline was replaced by undiluted blood; and further, that a heart changed back and forth would contract rhythmically or remain quiet according as it was filled with sodium chloride solution or with blood. Aubert's experiments demonstrated at least that

normal saline solution is not "indifferent" to living cardiac tissue in the same sense as is serum.

In these experiments I have tried in various ways to show what takes place when an apex strip is immersed in a bath of normal sodium chloride solution. In the earliest experiments 0.6 per cent sodium chloride was used, but later 0.7 per cent was thought to be more nearly isotonic. If a fresh strip saturated with blood be suspended in a bath of 0.6 per cent or 0.7 per cent sodium chloride solution it begins to beat rhythmically after a certain latent period. The length of the latent period, the height of the contractions, and the character of the rhythm and tone vary greatly in different experiments, but the following examples will serve as types.

TABLE I.

Table showing the great diversity of length of the latent period, rate, and maximal height of contractions produced by immersing a fresh ventricular strip of the terrapin in normal solutions of sodium chloride. The height given is the actual shortening of the strip in contraction.

| Number of experiment. | Latent period before contractions begin. | Maximal height of contractions. | Rate when the rhythm first becomes regular, together with the maximal rate in some examples. |
|-----------------------|------------------------------------------|---------------------------------|----------------------------------------------------------------------------------------------|
| 19 <i>b</i>           | 11 min.                                  | 1.3 cm.                         | 12 to 14 per min.                                                                            |
| 28 <i>b</i>           | 1 hr. 15 "                               | 0.15 "                          | 3 per min.                                                                                   |
| 29 <i>b</i>           | 1 " 30 "                                 | 0.2 "                           | 6, increasing to 10 per min.                                                                 |
| 31 <i>a</i>           | 2 " 35 "                                 | 0.82 "                          | 2.2 " " 7 " "                                                                                |
| 32 <i>a</i>           | 40 "                                     | 0.9 "                           | 6 " " 9 " "                                                                                  |
| 39 <i>c</i>           | 25 "                                     | 0.7 "                           | 6.5 " " 10 " "                                                                               |
| 40 <i>c</i>           | 1 " 00 "                                 | 0.9 "                           | 4 " " 8 " "                                                                                  |
| 46 <i>c</i>           | 2 " 3 "                                  | 0.6 "                           | 4 per min.                                                                                   |

The latent period may be only a few minutes, or it may be as many hours. It seems, in general, to be shortened when the bathing solution is renewed often. In some experiments, however, it was found to be long, even though the solution was often renewed. The height of the contractions varies much in different series, although in general it may be said that the longer the latent period the shorter the contractions when they do begin, and the shorter the time they are continued. I cannot at present give any evidence showing any quantitative relation of the rate and height to the latent period. It is to be noted that a sodium-chloride series, when once established, goes



through a regular series of changes in the rate and in the height of the contractions. The rate is almost always slow and irregular at the initiation of the series, becomes regular very quickly or after an interval of as much as twenty minutes, then slightly increases in frequency while decreasing in amplitude, until the contractions are reduced to the fraction of a millimetre in height. The height of the contractions in the series is at first submaximal, quickly increases to a maximum, and then very gradually decreases to a millimetre or less, passing into what has been designated as a state of saline exhaustion, the muscle remaining quiet in a relaxed state. If the muscle is left undisturbed in the solution while it is giving the series of contractions, the series decreases in height with beautiful regularity. But if during the series the solution be renewed, a slight increase in the height of the contractions generally occurs, and this is almost always followed by an increase in rate. When the strip has become quiet in sodium chloride solution, renewal of the solution has only a very slight effect, or else no effect at all, in recovering contractions in the strip. If there is any recovery, it is never more than a minute fraction of the original amplitude. A heart strip, saturated with blood, invariably loses tone when suspended in sodium chloride solution. The loss of tone is rapid at first, but becomes less rapid as the experiment

continually loses tone during the experiment, a fact characteristic of all sodium chloride experiments.

FIGURE 1. — Experiment 35 *a*, Feb. 3, 1898. Curve drawn by a strip of the apex of the terrapin suspended in one per cent sodium chloride solution. About one half the original size. Vertical magnification  $4/1$ . Time in minutes. A latent period of fifteen minutes occurs before contractions commence. The contractions begin at a slow rate, which quickly increases to a maximum of nine to ten per minute, and remains constant during the remainder of the experiment. The height of contractions becomes maximal in fifteen minutes, then rapidly and regularly decreases to less than a millimetre after seventy minutes. It will be noted that the muscle con-



progresses. The total loss of tone amounts to one fourth or one third the length of the strip in many experiments. In muscle strips that have previously been treated with solutions of other salts, sodium chloride produces slight or even no loss of tone.

With saline made slightly alkaline with sodium carbonate, I have never obtained a recovery of more than a small fraction of the original amplitude of the beat. This fact is noteworthy in comparison with results obtained on the saline "exhausted" frog's heart by Gaule,<sup>7</sup> Stiénon,<sup>6</sup> Martius,<sup>9</sup> and White.<sup>17</sup> According to my observations, it would seem that the heart-muscle of the terrapin treated with sodium chloride alone passes into a state from which it cannot be revived by alkaline sodium chloride solution. Although in consequence of sodium chloride treatment it gives smaller and smaller beats, and finally remains perfectly quiet in the relaxed state, still, as will be shown, the muscle strip may be thrown into most powerful rhythmic contractions at any moment, if only the proper inorganic salts be added to the saline solution. When the term "exhaustion" is used, therefore, in connection with the saline effect, it must be with the understanding that it applies only to the peculiar condition of quiescence produced by treatment with sodium chloride solution, a condition which might be designated more accurately simply as sodium chloride pause.

The amplitude of the contractions in a sodium chloride series is no criterion of the height of the contractions which the strip will give under the influence of other conditions, *i. e.*, solutions of the other inorganic constituents found in blood. In my experiments, some of the strips which I had condemned as from animals in bad condition, because they gave a poor sodium chloride series, when afterwards subjected to a bath of other inorganic salts gave contractions of unexpected amplitude. Strips cut from hearts which have previously been thoroughly irrigated with sodium chloride solution give only minute beats, or none at all, when suspended in a sodium chloride bath. They react, in fact, like the normal strip suspended in saline at the time when the saline series of contractions is nearly at an end, the exact similarity depending somewhat on the thoroughness of the irrigation. These strips, also, are not truly exhausted, as an experiment to be described later (No. 42) abundantly proves.

The great diversity of reaction to saline solutions exhibited by ventricular strips filled with blood is given at some length, because these are the variations in reactions often ascribed to the individual

peculiarities of the animal experimented upon. As a matter of fact, these reactions are only what might have been expected when we consider the cardiac rhythm in its relation to the other inorganic salts of blood. I hope later to suggest a possible explanation of these elements of difference.

If the strength of the sodium chloride to which a fresh muscle strip is subjected is varied, certain interesting phenomena are noticed. In two test experiments of three strips each, the strips in slightly hypertonic solution of sodium chloride began rhythmic contractions after a very short latent period, — a latent period shorter than was ever obtained from the fresh strips in what was assumed to be isotonic solutions, *i. e.*, 0.7 per cent. The strips in hypotonic solutions, on the other hand, were not constant in their behavior. One had an exceptionally long latent period; the other a shorter latent period than that of the control strip in isotonic solution.

TABLE II.

Variations of the strengths of the salts in relation to the latent period and to the rate and maximal height of the succeeding contractions.

| Number of experiment. | Per cent of NaCl. | Latent period. | Height of contractions was reduced to 0.1 cm. in | Maximal height of contractions. | Rate when the contractions first become regular. Also the maximal rate. |
|-----------------------|-------------------|----------------|--------------------------------------------------|---------------------------------|-------------------------------------------------------------------------|
| 35 <i>b</i>           | 0.7               | 38 min.        | 50 min.                                          | 0.8 cm.                         | 8 per min. — constant.                                                  |
| 36 <i>b</i>           | 0.7               | 24 “           | 70 “                                             | 0.6 “                           | 7, increasing to 10 per min.                                            |
| 35 <i>c</i>           | 1.0               | 15 “           | 70 “                                             | 1.15 “                          | 9.5 per min.                                                            |
| 36 <i>c</i>           | 1.0               | 3 “            | 42 “                                             | 1.0 “                           | 9, increasing to 11 per min.                                            |
| 35 <i>a</i>           | 0.4               | 3 “            | 42 “                                             | 0.7 “                           | 5 “ “ “ 8 “ “                                                           |
| 36 <i>a</i>           | 0.4               | 44 “           | 60 “                                             | 0.5 “                           | 6 “ “ “ 9 “ “                                                           |

The general cycle of events described above is characteristic of the saline curve of the normal strip. But if the strip is first treated with some other solution or kept in terrapin serum or blood for two or three days, or has been revived from a previous saline treatment and then again subjected to saline solution, its reactions present certain characteristic differences. Figures 2 and 3 illustrate these.

Figure 2 gives the reactions of two strips from the apex of the ventricle of the terrapin, the two from the same heart. Trace *a* begins at *x* with the immersion of the strip in serum twenty-one hours after suspension and following an unfavorable Ringer's solution.



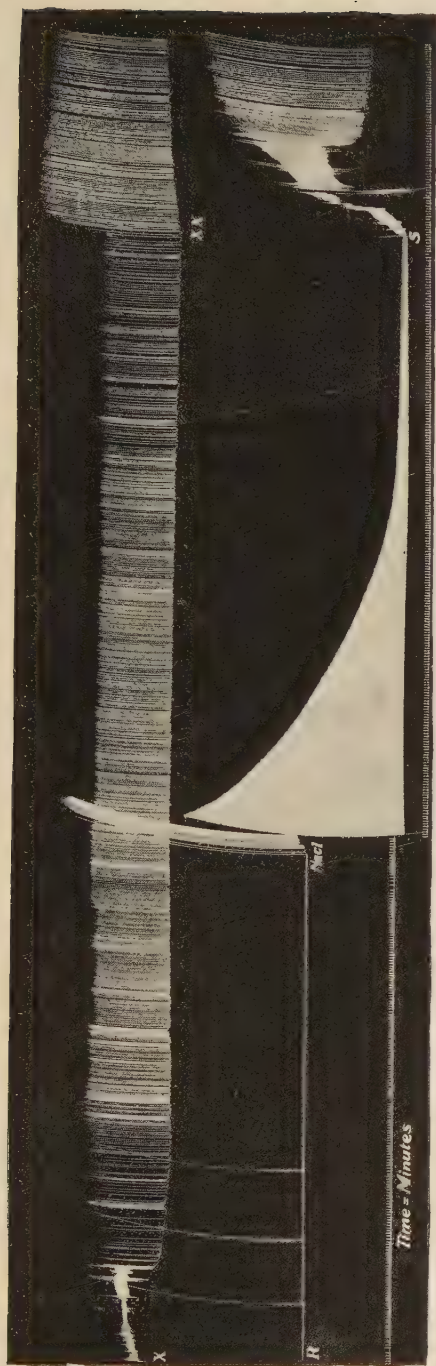


FIGURE 2. Experiment 30a and b, January 18, 1898. Vertical magnification, 4/1. Time in minutes. Reaction of two strips from the apex of the ventricle of a terrapin. *Trace a.* At *x*, immersion in serum; *xx*, the serum drawn off and Ringer's solution (NaCl 0.6%,  $\text{CaCl}_2$  0.026%, KCl 0.04%,  $\text{Na}_2\text{CO}_3$  0.003%) substituted. *Trace b.* A strip—quiet and relaxed in dilute serum, after 21 hours' suspension—was immersed at *R* in Ringer's solution (NaCl 0.6%,  $\text{CaCl}_2$  0.026%, KCl 0.05%,  $\text{Na}_2\text{CO}_3$  0.003%). At NaCl sodium chloride 0.6% substituted for the Ringer's solution (shortly afterward it was necessary to alter the position of the writing point.) *S*, immersion in dilute serum. The curve is about one half the original size.

The serum immediately produced fibrillation and tone increase, but after thirty-five minutes the strip suddenly relaxed and developed normal contractions at an irregular rate. The height remained nearly constant for seven hours. At *xx* the serum was drawn off and Ringer's solution (NaCl 0.6%,  $\text{CaCl}_2$  0.026%, KCl 0.04%, and  $\text{Na}_2\text{CO}_3$  0.003%) substituted. This was followed by a strong increase in the amplitude of contractions and a slight acceleration of rate. Trace *b* was recorded after a suspension of twenty-one hours. The strip was quiet and relaxed in dilute serum. At *R* it was immersed in Ringer's solution (NaCl 0.6%,  $\text{CaCl}_2$  0.026%, KCl 0.05%, and  $\text{Na}_2\text{CO}_3$  0.003%). No change was produced by the solution for three hours and ten minutes. The three isolated contractions indicate the state of the

muscle. A change to 0.6 per cent NaCl was immediately followed by a rapid rhythm, eight and more per minute. After four hours in sodium chloride solution the contractions were reduced to a small fraction of the original amplitude. That the activity of the strip was only suspended is shown by the effect of the dilute serum which was used at *S*. Serum produced strong increase in tone with fibrillation, which passed off gradually into well-coördinated contractions. Figure 3 gives the change in the experimental conditions following the effects shown in Fig. 2. In this experiment after thirty hours' suspension the strips *a* and *b* from terrapin No. 30 were immersed in 0.64 per cent sodium chloride solution; *a* had been previously immersed in Ringer's solution, *b* in serum diluted with 0.6 per cent sodium chloride. Strip

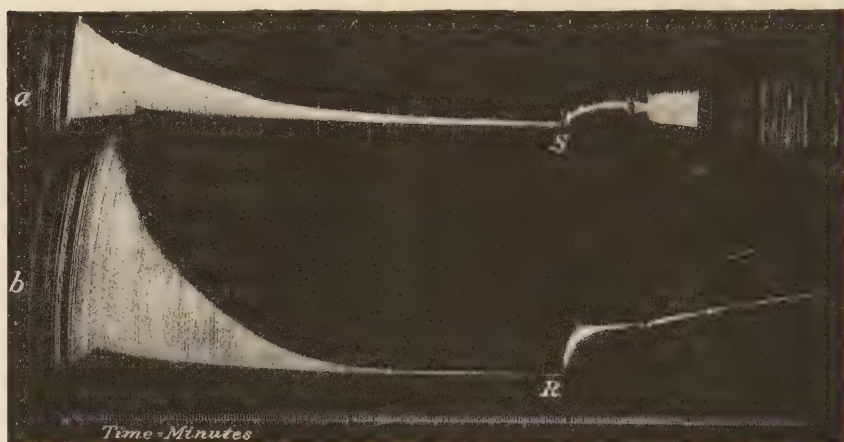


FIGURE 3. Experiment 30, *a* and *b*, January 18, 1898; vertical magnification 2/1; after thirty hours' suspension. Curves drawn by strips from terrapin ventricle immersed in 0.64 per cent sodium chloride solution. Strip *a* had been previously immersed in Ringer's solution; strip *b* in serum diluted with 0.6 per cent sodium chloride. *S*, immersion in serum; *R*, immersion in Ringer's solution. One half the original size.

*a* contracted at the rate of eleven to twelve and *b* at the rate of seven per minute. The similarity between these two traces and the corresponding one in Fig. 2 is very striking and suggestive. After two hours forty minutes in sodium chloride solution, serum produced in *a* an increase in tone with fibrillation, followed by good contractions. Ringer's solution produced in *b* a marked increase in tone with incoördinated contractions and fibrillations, from which the muscle did not recover until serum was again used.

First and perhaps most important of the characteristic differences illustrated by Figs. 2 and 3 is the shortening or entire absence of the latent period. If the strip has already been submitted to several changes and is still in condition to contract, then saline solution calls forth immediate contractions. Under these conditions also the contractions begin at a submaximal height, usually that of the contractions in the preceding solution, quickly increase to a maximum, and then regularly and uniformly decrease to complete disappearance, the muscle remaining inactive in a relaxed state. In these cases the maximal sodium chloride contractions are maximal for the muscle under *any* condition, a fact in sharp contrast to the submaximal contractions so often obtained from a fresh strip treated with sodium chloride solution. The rate is also much increased, in many experiments reaching twenty or more per minute, a rate too rapid to be distinguished in the records made on the slowly moving drum. The effects present the appearance of a very much heightened irritability, and the longer the muscle has been suspended the more striking is the result.

The rate in sodium chloride remains more nearly constant than in any other solution except, perhaps, very much diluted blood or serum. The decrease in the height of the contractions brought out in a heart strip by a second sodium chloride treatment is almost always perfectly regular and symmetrical. Such regularity in the response of the muscle to a definite salt in solution argues for a definite and regular series of changes in the muscle, either of a physical, *i. e.* osmotic, or of a chemical nature. I shall only briefly call attention here to certain facts which are brought out by this particular group of experiments.

It must be borne in mind that the muscle strip cut from a fresh unwashed ventricle is full of blood, that is, its muscle fibres are bathed in a liquid containing the numerous constituents of blood. When this strip is suspended in a solution of sodium chloride as nearly isotonic with blood as may be, it is to be presumed that diffusion of the salts other than sodium chloride immediately begins. Presumably osmotic currents of water and the diffusion of sodium chloride are reduced to a minimum, but all other diffusible constituents of the blood will tend to pass into the surrounding liquid. The more frequently this surrounding liquid is renewed the more rapidly the process is carried on. When contractions begin, as they do sooner or later, the rhythmical change in pressure greatly



aids the process. During the first few contractions the blood is squeezed out of the interstices of the strip in quantities sufficient to discolor the saline solution. When the whole heart is irrigated by saline solution before the strips of ventricle are cut the same process must necessarily go on during irrigation. In this case it is much more rapid, since the saline solution flows through the coronary system of blood vessels. The heart always contracts when irrigated, hence this mechanical aid to the washing out process is most effective in irrigating the whole heart.

Recalling, then, in the first place, that the apex ventricular strip cut from a heart previously irrigated and washed with saline solution will not contract when suspended in a saline solution, and secondly, that a heart strip filled with blood gives a rhythmic series of beats, diminishing rapidly to zero when suspended in a saline solution, and thirdly, that a strip revived by other solutions and again surrounded by sodium chloride solution repeats essentially the same process, my results may be briefly summarized as follows:

1. Sodium chloride in solution in distilled water will sustain the ventricular muscle of the terrapin in rhythmic contractions for a brief time only, one to two hours.

2. The ventricular muscle is not exhausted by sodium chloride solution, although the decreasing amplitude of the series of contractions simulates exhaustion.

3. Sodium chloride solutions stimulate a heart strip to a brief series of contractions of increasing rhythm and decreasing amplitude.

4. A sodium chloride bath does not always develop maximal contractions in a heart strip.

5. Sodium chloride solution produces marked loss of tone in a fresh ventricular strip.

**Calcium chloride.**—Calcium exists in the blood presumably in combination and as free salts or the corresponding ions. Quantitative analyses of the calcium present in the blood of the terrapin were made in several instances. A large terrapin was bled for such an analysis, and the blood kept on ice a day or more until the corpuscles had settled. From fifty to sixty cubic centimetres of clear amber-colored plasma were obtained from a single terrapin. The plasma to be analyzed was siphoned off into a standardized graduated burette, measured amounts drawn into large centrifugalizing tubes, ammonia added to slight excess, and the calcium precipitated with ammonium oxalate. The precipitate was now left to settle, or else

was thrown down by means of a centrifugal machine, washed in distilled water, redissolved in weak hydrochloric acid, reprecipitated, washed until free of chlorine, dried, and finally heated in a platinum crucible until the weight remained constant. Duplicate analyses were made and the results are expressed as grams of calcium oxide in 100 c.c. of plasma. Each tube in Experiment I contained 24 c.c., in Experiment II, 25 c.c. of plasma.

TABLE III.

*Determination of calcium in the plasma of the slider terrapin expressed as calcium oxide in 100 c.c. of plasma.*

**Experiment I.** December 4, 1897.  $a = 0.0126$  gram,  $b = 0.0126$  gram.

**Experiment II.** December 11, 1887.  $a = 0.0133$  gram,  $b = 0.0141$  gram.

Mean of four determinations, 0.0131 gram.

This determination is in fair agreement with the results of Gerlach,<sup>20</sup> secured by the same method from dog's serum, namely, 0.014 and 0.0145 gram CaO in 100 c.c. serum, and also with determinations of the calcium in sheep serum made by Dr. Howell in this laboratory by a volumetric method with potassium permanganate, which gave as the mean of two analyses 0.0124 CaO in 100 c.c. of serum.

The mean of the determinations in Table III expressed as calcium chloride is 0.026 gram per 100 c.c. of plasma. This amount was considered as the amount normal to the blood. Chloride salts were used in all the artificial inorganic salt solutions in order to reduce the effect of the acid radical to a constant. One would expect the amount of calcium to vary in different individuals. It is possible, too, that some of the combined calcium may separate off, and if so the above amount would be too large. It will, however, serve as the most available constant in the study of the effects of the calcium in the blood in its relation to the development and maintenance of contractions in the isolated heart strip. In 1883 Ringer<sup>10</sup> first demonstrated the great importance of this element in the activity of the frog's heart. Since that time numerous experimenters have extended and enlarged our knowledge of the physiological importance of calcium in the animal organism. At the present time it is generally recognized that calcium in some form plays an essential part not only in the activity of muscle but also in the clotting of blood, the coagulation of milk, etc.

Ringer demonstrated that calcium does not act alone in maintain-

ing the rhythm of the frog's heart, but that it must be antagonized by potassium salts. I will here first discuss briefly the effects of calcium and potassium salts as such, and later take up their relation to other salts. Isotonic strengths of calcium are imperfectly borne by the cardiac muscle, hence calcium effects must be studied in combination with some other isotonic solution. Of these I have used isotonic solutions of sodium chloride, dextrose, and urea. The last seems injurious, and no contractions have been observed in it. My most reliable calcium effects are, therefore, those obtained in combination with saline effects.

*Calcium chloride alone.* — Calcium chloride in distilled water in approximately isotonic solution when applied directly to a heart strip throws the muscle into strong tone. No rhythmical contractions are given off for five minutes, — the longest time a strip has been submitted to this excessively strong solution. When the excess of calcium is removed by washing the strip with 0.7 per cent sodium chloride solution, a series of very rapid contractions immediately starts up. The rhythmic contractions are superposed upon a state of strong tonic shortening. No permanent injurious effect follows the use of the strong calcium solutions. If, on the other hand, calcium chloride of the strength found in the blood is applied to a heart strip that is quiet but in good condition for contracting, its application is followed immediately by a rapid and regular series of contractions. The contractions decrease rapidly in height for five minutes, — the longest time a strip has been submitted to this hypotonic solution. In these contractions the relaxation phase is much shortened and the lever does not return to the base line.

The experiments with solutions of calcium chloride alone are not very satisfactory although suggestive. Isotonic solutions on the one hand contain a deleterious amount of calcium, and on the other hand normal strengths of calcium are so strongly hypotonic to the muscle that this physical factor is doubtless a predominant one in determining the results.

*Potassium chloride.* — Potassium chloride, like calcium chloride, is deleterious when applied to the muscle of the heart in isotonic solutions. Its effects are also complicated by physical phenomena when it is applied in solutions of a strength found normally in the blood. When one per cent potassium chloride solution was applied to a heart strip which was previously contracting rhythmically in dilute serum the heart strip quickly gave one or two spasmodic contractions



and then remained quiet in a condition of tone. Afterward, when the excess of potassium was removed by washing the strip with 0.7 per cent sodium chloride, no contractions were developed but the tone spasm passed off. Dilute serum again established a rhythm after a short latent period, and the rhythm appeared perfectly normal in character. From this it will be seen that excessive doses of potassium as well as of calcium salts do not produce a permanent poisonous effect on the ventricular strip when applied for short periods.

In more dilute solutions, *i.e.*, in solutions approximately normal to the blood (0.03 to 0.04 per cent), as determined by physiological reaction and by analyses reported for the blood of other animals, potassium chloride applied to a contracting strip produced quiescence after a few contractions which decreased rapidly in amplitude to complete disappearance. Tonic shortening also followed the use of this hypotonic solution.

#### SODIUM, POTASSIUM, AND CALCIUM SALTS IN COMBINATION.

**Calcium chloride in isotonic solutions of sodium chloride.**—If calcium chloride, in an amount normal or subnormal to the blood, be added to isotonic solutions of sodium chloride, certain important results are obtained. This mixture of salts, applied to a fresh, unwashed heart strip, produces a series of contractions after a very short latent period. The series resembles, in general features, the series of contractions given by a control strip in saline alone. But the rate is more rapid in the strip in sodium and calcium chloride solution, and the contractions are maximal from the first, while in the pure sodium chloride solution contractions are usually not maximal for some minutes after the series is inaugurated. The most characteristic effect of the calcium when added to sodium chloride solution is the prevention of perfect relaxation after each contraction. There is a strong rise of the base line instead of the gradual fall so characteristic of the sodium chloride series. If the amount of calcium chloride used on a fresh strip is large, say 0.04 per cent, the rise occurs very soon after the strip begins automatic contractions. The rise begins immediately on the application of the solution when applied to a strip that is quiet after a previous sodium chloride treatment. When a strip ceases to beat in a sodium and calcium chloride mixture it ceases in a state of tone. If the amount of calcium

is small, 0.01 per cent, then the increase in muscular tone is only slight. Calcium chloride in solutions of sodium chloride never more than slightly revives a heart strip after it has ceased to contract in a solution of sodium chloride alone.

**Calcium chloride in isotonic solutions of dextrose.** — Calcium chloride in amount normal to the blood, 0.026 per cent, when applied in isotonic solutions of dextrose to a heart strip previously beating in serum, or in serum diluted with saline, immediately calls forth a series of rapid contractions, together with a strong increase in tone. When the dextrose alone is applied to the heart strip, it calls forth a similar, though less rapid, series of contractions. It is questionable, therefore, just how much of the above effect is due to dextrose and how much to calcium, a point which requires further investigation.

**Calcium chloride in isotonic solutions of urea.** — Calcium chloride in isotonic solutions of urea produced no contractions in the muscle strip. Urea itself seems injurious to cardiac muscle when applied in isotonic strength, for even serum produces only slight or no recovery after its use.

**Potassium chloride in isotonic solutions of sodium chloride.** — When a fresh muscle strip is immersed in a solution of 0.03 per cent potassium chloride in isotonic solutions of sodium chloride, either no contractions at all are developed, or, if developed, the contractions are extremely minute, and make their appearance only after an extremely long latent period. The contractions recorded in the exceptional cases occur very irregularly, and are only a small fraction of the height (one ninth in one experiment) of the contractions given after a change to a solution of different composition. These results are in sharp contrast with the behavior of a strip surrounded by sodium chloride alone, or by sodium and calcium chloride solution. Strips in solutions of sodium and potassium chloride almost always show an excessive loss of tone, a result just the opposite to that of strips in solutions of sodium and calcium chloride. Solutions of sodium and potassium chloride have no effect in reviving activity in a strip that has ceased to beat in sodium chloride alone.

Ringer<sup>10</sup> and his students have taken a prominent part in investigations concerning the action of potassium salts on the animal body and on the heart. They have shown that potassium salts applied to the frog's heart produce a slowing of the rate, much dilatation,

and ultimate cessation of the rhythm. Ringer was also the first to show the necessity of this salt in antagonizing the excessive stimulating effect of calcium salts. We will now turn to this phase of the subject as applied to the terrapin heart strip.

**Sodium, calcium, and potassium chlorides in isotonic solution.** — The wonderful sustaining power of sodium, potassium, and calcium salts in solution was first pointed out by Ringer<sup>10</sup> in 1883. In the proportions used in his original formula the solution was shown to revive the frog's heart, and to sustain it in rhythmic contraction for several hours. Ringer afterward substituted the tribasic phosphate for the calcium chloride; but, in so far as the cardiac muscular strip is concerned, I have obtained quite satisfactory results with the chloride.

In my experiments I have striven to secure a proportion among the above inorganic salts that would give the effects on an isolated cardiac strip most nearly approaching that of blood. The facts already pointed out by Ringer and his students, and by Howell and Cooke,<sup>15</sup> seem to indicate that if one could exactly imitate the composition of the blood as regards the inorganic constituents, it would be possible to secure, approximately, the same effects from blood and from the artificial preparation of inorganic salts in so far as the isolated strip is concerned.

By keeping the amount of calcium in isotonic sodium chloride solution constant and equal to the mean of the two analyses of the terrapin plasma, and by varying the amount of potassium, a solution was soon determined which gave fairly constant results. With this solution results were obtained that closely approximated the effects of a serum bath on the ventricular strip. The amounts of the three salts in this solution were: sodium chloride, 0.6–0.7 per cent; calcium chloride, 0.026 per cent; potassium chloride, 0.03–0.04 per cent; a trace of sodium carbonate was sometimes added. This solution of the inorganic salts of blood will keep a heart strip alive and in condition to beat for a very long time, 72 hours and more. The inorganic solution does not keep the strip in as good condition as does blood or serum, but the parallelism between the two is very striking. The following experiment exhibits the close relation in the action of the above inorganic salt solution and of serum on ventricular strips.

*Experiment 34, January 29, 1898.* The apical third of the ventricle was cut into thin strips which were suspended in heart tubes while still saturated with

blood. Strip *a* was immersed in pure serum; strip *d* in a bath of Ringer's mixture (0.6% NaCl, 0.026 CaCl<sub>2</sub>, 0.04% KCl, and a trace of Na<sub>2</sub>CO<sub>3</sub>).

*a*. The serum strip gave a few beats when first suspended, but in a few minutes became quiet in a relaxed state. Single contractions occurred at long and irregular intervals during ninety-six hours, when the strip was changed to a bath of 0.6 per cent sodium chloride. Sodium chloride solution called forth the customary series of contractions.

*d*. The strip in Ringer's solution remained quiet in a relaxed state, giving only occasional single contractions during seventy-six hours. The contractions which were recorded during a portion of this time did not average more than one per hour. At the end of seventy-six hours the strip was changed to a bath of 0.6 per cent sodium chloride solution, and a rapid series of contractions was produced at once (see Fig. 4). While the maximal contractions of this series were only 0.5 cm. high, about one half the height of those of strip *a* in saline, still the series resembled a typical sodium chloride series in that the contractions rapidly declined in amplitude until the strip remained quiet in the diastolic state.

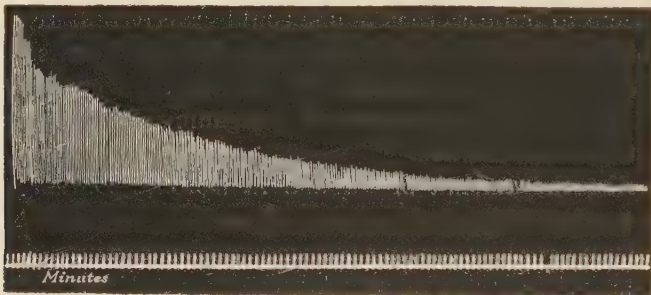


FIGURE 4. Experiment 34 *d*, January 29, 1898. Vertical magnification 4/1. Time trace marks minutes. To illustrate the effect of a 0.6 per cent bath of sodium chloride solution on a strip of terrapin's ventricle after seventy-six hours' suspension in Ringer's solution. When the sodium chloride was introduced, regular contractions began at once, as the above shows.

The following is a second example of the similarity of action between the solution of inorganic salts and pure serum:—

*Terrapin 45, February 28, 1898.* The apex of the ventricle saturated with blood was cut into thin strips and suspended in heart tubes, *a* in Ringer's solution, *b* and *c* in air. Strip *b* was moistened by occasional drops of Ringer's solution (0.7% NaCl, 0.02% CaCl<sub>2</sub>, and 0.03% KCl), and *c* with drops of serum.

Strip *a*, after a latent period of twenty minutes, gave contractions that were



perfectly normal in character but very irregular in rate. The rate averaged one to four per minute. Neither *b* nor *c* contracted for thirty consecutive hours, but when moistened with sodium chloride solution both strips gave contractions that were complete and apparently normal in character.

This experiment again demonstrates that solutions of inorganic salts, as well as serum, are able to keep the ventricular strip in good condition, and that they, like serum, will not necessarily stimulate the strip to rhythmic contractions.



FIGURE 5. Experiment 42 *b*, February 14, 1898.

Vertical magnification 4/1; time trace marks minutes. A ventricular strip from the apex of a terrapin's heart, moist with Ringer's solution, and contracting in a moist chamber. See protocol of Experiment 42. This strip, at the end of twenty-two hours' suspension in air, and still moist with Ringer's solution containing 0.7% NaCl, 0.026%  $\text{CaCl}_2$ , and 0.03% KCl, contracted only at long intervals, giving only sixteen contractions in the last two hours. When the strip was moistened with Ringer's solution, with the calcium chloride increased to 0.04%, the above beautifully regular contractions began and continued for one hour fifty minutes without interruption. Rate, four per minute. Compare with Fig. 6.

This experiment is also important in its bearing on another experiment to be given presently, Experiment 42. The fact that one heart strip immersed in a given inorganic solution beats while another strip from the same heart does not beat when in air saturated with water vapor, and moistened only occasionally with the same inorganic solution, indicates some process more favorable to the development of contractions in the presence of the liquid bath. This cannot be ascribed to any hindering effect due to the presence of the air. In fact, one would expect the better aëration of the tissue surrounded by air to facilitate the development of contractions rather than to delay contractions. This point is brought forward and emphasized at this

time; for if strips be first washed out in sodium chloride solution, then bathed for a few minutes with Ringer's solution, and suspended in air, they may become as much as ten times more active than strips kept in a constant bath of the same solution.

The particular results to be expected when a fresh strip of ventricle containing the normal amount of blood is subjected to a bath of sodium, calcium and potassium chloride solution, so far as my experiments go, depend upon the relative proportions of the calcium and potassium chlorides. If these salts are in the proportions of 0.026 per cent calcium chloride to 0.03 per cent potassium chloride, a few good contractions at a very slow and irregular rate may result. If this ratio is changed by increasing the calcium or by decreasing the potassium, then the contractions are increased in frequency (Figs. 5 and 6). But if the calcium is diminished or the potassium increased, few contractions are developed, or none at all. Experiment 34 *d* gave fifteen contractions in the first forty-six hours. The solution used contained 0.6 per cent sodium chloride, 0.026 per cent calcium chloride, 0.04 per cent potassium chloride, and 0.003 per cent sodium carbonate.

If the muscle strip is cut from a heart previously irrigated with saline, or from one that has been subjected to the influence of some other artificial solution, the effect produced on the strip by Ringer's solution varies according to at least two factors: first the relative amounts of the calcium and potassium chlorides in the Ringer's solution, and second, the composition of the solution to which the strip has previously been submitted, together with the time the latter has acted.

**Ringer's solution following sodium chloride solution.** — A ventricular strip treated with sodium chloride, 0.6 to 0.7 per cent, until it ceases to contract, is stimulated to a strong and regular series of contractions when bathed in a suitable Ringer's solution. The amplitude and general character of the contractions of a strip revived in this way can only be compared with the contractions of a strip revived by serum or by serum diluted with saline. By moistening the strips with Ringer's solution, in which the proportion of calcium chloride is increased from time to time, strips may be kept beating for sixty hours and more after the apparent exhaustion in sodium chloride. In one experiment, No. 42, three companion strips were in this way kept contracting for fifty-one and a half, sixty-two, and forty-two hours.

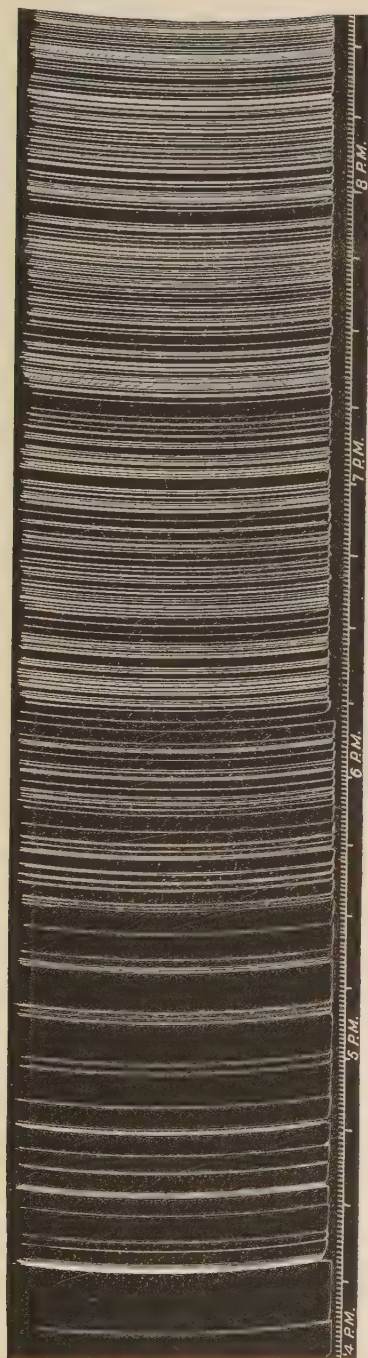


FIGURE 6. Experiment 42 *a*, February 14, 1898. Vertical magnification  $4/1$ . Time trace in minutes. To illustrate the effect on a quiet strip of increasing the amount of calcium chloride in the Ringer's solution used. The curves are about one half the original size.

The ventricular strip from which this trace was obtained was from a terrapin's heart that had been washed free of blood with 0.6 per cent sodium chloride solution, then suspended in a Ringer's solution until a strong rhythm was established, and finally left contracting in moist air. The contractions were always full, but the rate became slower and more irregular, and ceased at eighteen hours after suspension. The strip was now moistened with stock Ringer's solution, then with a solution containing successively 0.03%, 0.04%, and 0.05% and finally 0.06% calcium chloride. The last moistening occurred at the twenty-seventh hour of suspension. In twelve minutes after the last moistening contractions began again at an irregular rate. The contractions increased in frequency, and became fairly regular in four hours, as the above trace illustrates. See also Fig. 5.

In another experiment, 33 *a* and *d*, strips immersed in Ringer's solution contracted rhythmically and normally for twenty-seven hours, when the record was lost. After forty-two hours these strips were quiet, but apparently in good condition for contraction, as both strips were immediately revived, and gave fine contractions when changed to serum. The recovery in both these strips in serum closely resembles the recovery of contractions in strips treated with sodium chloride, and then changed to Ringer's solution or to extract of serum. This suggests a recovery due to the effects of the better combination of salts in the terrapin's own serum. At least, I am strongly inclined to believe that the revival in this case is due to the fact that serum contains the conditions or sub-

stances most favorable to the complete using up of the contractile material still in the strip.

If the strip is changed from sodium chloride to Ringer's solution while the contractions are small and regular in rate, the revival of complete contractions is very prompt. The contractions become maximal in a very few minutes, and the rate is often perfectly regular for a time. Afterward, however, the rate becomes slower and very irregular, a result perfectly analogous to the serum effect under the same circumstances.

On the other hand, if the contractions have entirely ceased in sodium chloride, and the muscle has remained in the solution from one to several hours, and then is suddenly subjected to a bath of Ringer's solution, the result is quite different. Under such circumstances the muscle is thrown into immediate fibrillation, from which it never recovers while in the Ringer's solution. This is apparently strictly a calcium effect. For if, instead of the ordinary combination of salts in the Ringer's solution, a solution containing a reduced amount of calcium is first applied to the strip and the calcium gradually increased to the normal amount, good contractions are produced and fibrillation avoided.

Muscle strips left in sodium chloride solution a long time invariably go into strong contracture when changed to ordinary Ringer's solution, whether they beat or not. If fibrillation results, the tonic shortening amounts to one third or even one half the amount of

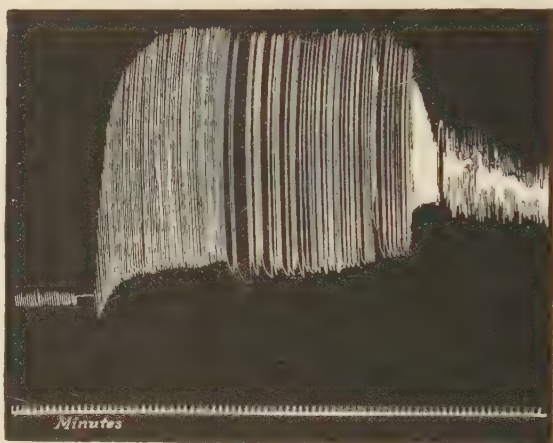


FIGURE 7. Experiment 46 *a*, March 2, 1898. Vertical magnification, 4/1. Time trace marks minutes. To illustrate the effect of a favorable combination of the salts in Ringer's solution in reviving contractions in a strip of the terrapin ventricle after its contractions in sodium chloride were reduced to a fraction of a millimetre in height. On immersing the strip of ventricle in Ringer's solution the contractions quickly increase in amplitude to a maximum, become irregular in rate after twenty minutes, and pass into fibrillation after about fifty minutes. Seven ninths the original size.



shortening previously produced in the same muscle during a normal contraction. If the saline strip is treated with a solution of sodium chloride and potassium chloride before the Ringer's solution is used, contracture is diminished and fibrillation delayed, though not necessarily avoided.

In certain experiments normal Ringer's solution following sodium chloride solution produced an excellent recovery for a time, but later the strips went into a state of fibrillation. Perhaps it would be better to describe this state as one of incoördinated contractions followed by fibrillation. Such strips while giving regular normal contractions in Ringer's solution suddenly show independent rhythm in two or three parts of the strip at the same time, and then gradually pass into fibrillation.

In a single example, a strip contracting in serum after repeated revivals from sodium chloride solution gave much larger beats when changed from serum to Ringer's solution (see Fig. 2 *a*). The height increased from 0.6 cm. to 1.05 cm. The rate, which was irregular both before and after the change, was increased from an average of 0.3 to 2.8 per minute. This effect followed at once, and is presumably due to the stimulating effect of the calcium.

**Ringer's solution following a solution of sodium and potassium chloride.**—A normal strip that has been kept from contracting by a solution of potassium in sodium chloride gives normal rhythmic contractions when the bath is changed to Ringer's solution. If the amount of potassium chloride in the sodium and potassium solution has been great, say 0.04 to 0.05 per cent, the contractions called forth by the bath of Ringer's solution may be at a very slow rate, or may appear in groups at a good rate, but with the groups separated by long periods of rest, during which the muscle remains in a perfectly relaxed state. Ringer's solution after a solution of sodium and potassium chloride is not followed by tone shortening, such as occurs after sodium chloride alone.

If a strip in a sodium and potassium chloride solution be transferred first to a Ringer's solution having a reduced amount of calcium, and later the calcium be increased, no contractions are developed until the amount of calcium in the Ringer's solution is increased to at least the normal amount found in blood.

Of the strips which were transferred to a bath of ordinary Ringer's solution after treatment with a solution of sodium and

potassium chloride, two developed full normal contractions in from five to ten minutes, and after forty to sixty minutes each strip fibrillated. Presumably this result was due, in each case, to the strong stimulating effect of the calcium, which was fully counteracted by the potassium until the calcium had time to diffuse more completely into the strip.

#### EXTRACT OF THE EVAPORATED RESIDUE OF SERUM.

Terrapin serum was dried over a water bath, and the dried residue pulverized and extracted with distilled water. The filtered extract was evaporated to dryness and a second extract made and diluted to the original volume of serum. The second extract gave

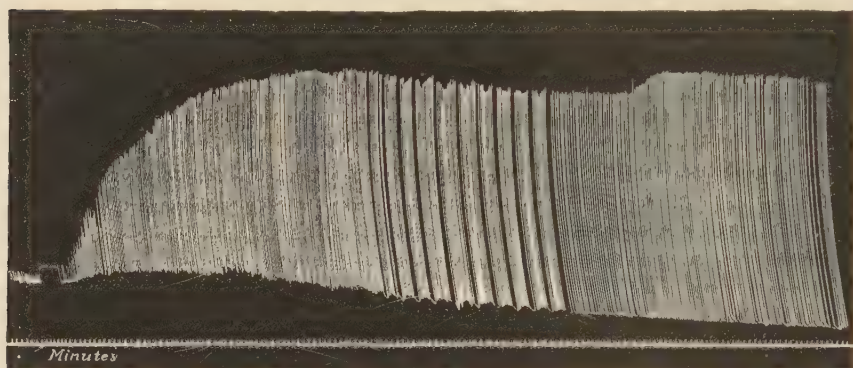


FIGURE 8. From Experiment 46*c*, March 2, 1898; vertical magnification 4/1. Time trace marks minutes. To illustrate the effect of an extract of the dried residue of terrapin serum in reviving full contractions in a strip of ventricle that had almost ceased to contract in sodium chloride solution. Companion to the tracing shown in Fig. 7.

only a very faint color change when tested for proteid by the xanthoproteic test, and no proteid was detected by Millon's reagent. Careful testing for sugar with Fehling's fluid gave a questionable precipitate. This extract was compared with blood and with Ringer's solution in its reviving effects on the heart strip after saline treatment. The close similarity in the results is shown in the following protocol and the accompanying Fig. 8.

*Experiment 46, March 2, 1898.* Three ventricular strips cut from the normal heart were first immersed in saline until the contractions produced in each were reduced to 0.04 cm. and less in height. Strip *a* was transferred

from saline to Ringer's solution (0.7% NaCl, 0.026%  $\text{CaCl}_2$ , 0.03% KCl). Strip *b* was transferred to serum, and *c* to an extract of the salts from the evaporated residue of serum.

*Strip a.* So soon as Ringer's solution surrounded the strip, contractions began and continued at a regular rate but rapidly increased in height with each successive contraction until in ten minutes they had increased from 0.04 cm. to 0.8 cm. The rate remained regular for twenty minutes, then became irregular for thirty minutes. After fifty minutes the strip gave a series of very rapid contractions and then went into fibrillation, from which it did not recover. (Fig. 7.)

*Strip b.* In serum; was accidentally lost.

*Strip c.* The extract of the salts of serum produced a rapid recovery of the amplitude, but not so rapid a recovery as that of strip *a* in Ringer's solution. The height increased in thirty minutes from 0.03 cm. to 0.9 cm. The rate for the first hour was slightly irregular, being from four to five per minute. After one hour in serum extract Luciani's periods appeared. There were ten of these groups in forty minutes. In every group the contractions began at a slow rate, increased to a maximum rate, then became slow again. A pause of from thirty to sixty seconds intervened between each two of the several groups. The rate following the groups was perfectly regular — two per minute — for several minutes. At the end of two hours and thirty-five minutes the contractions suddenly ceased entirely. With the exception of three single contractions the strip remained quiet in relaxation until transferred to 0.7 per cent saline, when it gave the customary saline series. The last contractions of the serum extract series were 1.03 cm. high. This series ended very much like that of a slightly diluted serum.

On the whole, the serum extract revived the strip *c* more normally, that is, more like serum, than did Ringer's solution in the case of strip *a*. The after effects were also more like serum, as shown in other serum experiments. Another experiment with serum extract, on strip *a* of Exp. 46, bears out this view. Serum extract was used on the strip when in fibrillation after long exhaustion, and at once produced increased activity. After seventy minutes the fibrillating strip relaxed suddenly, and began slow and irregular, yet apparently perfect contractions. The relaxation was, however, only partial in this case. This was the only instance in all my experiments in which I obtained this type of recovery from fibrillation with anything but pure serum.

## ON THE WORK GIVEN OFF BY A CARDIAC STRIP AND THE SOURCE OF THE ENERGY OF CONTRACTION.

The amount of energy liberated by a heart strip bathed in an inorganic salt solution has been so great in many experiments as to raise the question as to the source of the energy-giving material.

In Experiment 33 *a* a fresh strip that had been bathed in 0.6 per cent sodium chloride until no further beats were produced was changed to a bath of Ringer's solution. It almost immediately gave contractions which were normal in general character, and higher and stronger than the largest contractions obtained from the strip while in the sodium chloride bath. The rate, however, was slow and irregular. The contractions of this strip in Ringer's solution increased in height and improved in rate, and in twenty-seven hours gave 1670 gramcentimetres of work. The record was lost from the twenty-seventh till the forty-second hour, at the end of which time the quiet muscle was transferred to serum. This partial record strongly suggests the view that the amount of energy developed by the muscle strip is greater than can be accounted for by the proteid constituents of the blood in the muscle. To test this question more thoroughly, Experiment 42, given in detail below, was tried.

*Experiment 42, February 14, 1898.* A terrapin's heart was washed as free as possible of blood by continuous irrigation with 0.7 per cent sodium chloride solution. The washing was facilitated by cutting the coronary veins and by gentle massage applied to the ventricle. Irrigation continued one hour, and at the end of that time the ventricle was still giving feeble contractions. Thirty minutes were consumed in preparing, weighing, and suspending three ventricular strips. They were, therefore, bathed in 0.7 per cent solution of sodium chloride an hour and thirty minutes. The strips in the heart tubes were next surrounded by a bath of Ringer's solution (0.7% NaCl, 0.026%  $\text{CaCl}_2$ , and 0.03% KCl) for thirty minutes, during which time strong contractions were established in each strip. The Ringer's solution was next drawn off, leaving the strips in moist air. They were occasionally moistened with a momentary bath or with drops of Ringer's solution. The three strips were kept thus in air fifty-one and a half, seventy-two and two thirds, and fifty hours respectively.

*Strip a.* This strip was kept moistened with Ringer's solution, of the composition given above, for seventeen hours twenty-five minutes. The contractions were comparatively rapid at first, about one half the total number of



contractions occurring during the first seven hours. During this first period of suspension the rate slowly decreased, so that at the end of eight hours the strip was beating with an irregular rhythm and with a tendency to grouping. The rate temporarily increased after each remoistening of the strip. The amplitude began with 0.9 cm. actual shortening during a contraction, and slowly but steadily increased to 1.38 cm. in twenty-six hours; it remained above 1.0 cm. for forty-five hours. The contractions were apparently always complete whether the rate was fast or slow. The record was lost from thirty minutes past the tenth hour to fifteen minutes past the seventeenth hour of suspension in air. At the end of this time strip *a* was not contracting. Remoistening with the usual Ringer's solution produced no contractions. Ringer's solution with increased amounts of calcium chloride was then applied to the strip. During eight hours (the seventeenth to the twenty-fifth) the calcium chloride was increased successively to 0.03, 0.04, 0.05, and 0.06 per cent. With Ringer's solution containing 0.06 per cent calcium chloride, the strip again began to contract and continued to do so for twenty-six hours thirty minutes, with but one remoistening. The contractions, at first slow and irregular in rate, increased from forty the first hour (twenty-sixth of the experiment) to one hundred and sixty-one the sixth hour (thirty-second of the experiment). During the succeeding night the room temperature decreased to 4° C. and both rate and height of contractions were somewhat reduced, but both recovered again the next morning.

The forty-fifth hour after suspension in air the contractions began slowly to decrease in amplitude, reaching a zero in six hours, thirty minutes, *i. e.*, after a total time of activity in Ringer's solution of fifty-one hours and thirty minutes. The proportion of calcium chloride was again increased, but no contractions were produced even with 0.10 per cent.

A bath of horse's serum applied to the strip at fifty-one and a half hours was followed immediately by a series of contractions lasting for sixteen hours. The rate began with five per minute, but varied greatly at different times and the contractions were often quite incoördinated. In serum the height was 0.35 cm. at first, about one third that of the normal contractions of this strip, but decreased to zero in sixteen hours, *i. e.*, the sixty-seventh hour of the experiment. Minute contractions, 0.02 cm., were still obtained by diluted serum at the seventy-fourth hour.

*Strip b.* This strip was continued in air for seventy-one hours. For the first ten hours the record had all the general features of *a*, except that the contractions were at a somewhat slower and more irregular rate and were higher. Luciani's groups with well-marked "stair-case" occurred from the ninth to the twenty-second hour. Only sixteen contractions occurred from the twentieth to the twenty-second hour of suspension in air. Ringer's solution with the calcium chloride increased to 0.04 per cent was used to wet the strip at the twenty-second hour, and was immediately followed by tall, regular

contractions for a period of over two hours. These contractions ceased quite abruptly. After a long pause — at the twenty-fifth hour — the strip was wet with drops of Ringer's solution containing 0.05 per cent calcium chloride. Immediately Luciani's groups appeared, with long intervening pauses. The groups became more and more frequent, and after three hours they passed into an irregular rhythm which continued twenty-five hours, thirty minutes. During the night the rate and height were both decreased by the low temperature, as in *a*. The calcium chloride was again increased in the Ringer's solution to 0.06 per cent, and at fifty-one hours, thirty minutes, the strip was given a two minutes' bath. The only effect was a slight increase in rate. Following the fiftieth hour of suspension the height of the contractions slowly decreased for twelve hours from 0.98 cm. to zero. Bathing the strip with Ringer's solutions containing 0.06 and 0.08 per cent calcium chloride, respectively, brought out contractions of good rate but only a millimetre in height (not recorded in the table below). At seventy-two hours strong electrical stimulation produced no observable contractions. At seventy-two hours, forty minutes, the strip was immersed in a bath of horse's serum, which produced contractions only 0.02 cm. to 0.03 cm. in height, *i. e.*, no recovery. The serum in this case, therefore, failed to cause an improvement in contractions. The experiment ended at the seventy-fourth hour of suspension in air.

*Strip c.* This strip in air contracted rapidly for a time but gradually became slower during twenty-four hours, forty-five minutes. Its contractions decreased in rate more gradually than in *a* or *b*, and Luciani's groups were not so prominent. Wetting the strip with Ringer's solution (calcium chloride 0.04 per cent) at twenty-four hours, forty-five minutes, was followed by an increase in the rate. After thirty hours, fifteen minutes, increase of calcium chloride to 0.05 per cent produced a slight increase in the rate. The height of the contractions slowly decreased from 0.69 cm. at the thirty-first hour to zero at the forty-second hour (see table below). The number of contractions for the last ten hours was very small. Further increase of calcium produced in each case comparatively rapid contractions, but no recovery of the height; thus, at the forty-second hour, the height of the contractions immediately following calcium chloride solution 0.06 per cent was 0.14 cm.; at the forty-fourth hour, following 0.08 per cent calcium chloride solution, it was 0.18 cm.; and at the forty-sixth hour, minute contractions were observed after 0.1 per cent calcium chloride. At the fiftieth hour the strip was given a bath of terrapin serum which produced contractions only 0.15 cm. high. No strong full contractions resulted.

I have made the amount of work given by these three strips of ventricular muscle while contracting automatically in a purely inorganic diet the basis of a series of calculations, in order to express the results in a way that admits of comparative study.

TABLE IV.

Tabulated results of Experiment 42 *a*, *b*, and *c*.

| Time of suspension in air. | Number of contractions per hour. |     |     | Mean height of contractions during the hour = actual shortening of the muscle in centimetres. |      |      | Work done during one hour expressed as gramcentimetres. |     |     |
|----------------------------|----------------------------------|-----|-----|-----------------------------------------------------------------------------------------------|------|------|---------------------------------------------------------|-----|-----|
| hour                       | a.                               | b.  | c.  | a.                                                                                            | b.   | c.   | a.                                                      | b.  | c.  |
| 0-1                        | 512                              | 305 | 440 | 0.90                                                                                          | 1.30 | 1.00 | 461                                                     | 396 | 440 |
| 1-2                        | 564                              | 265 | 420 | 0.90                                                                                          | 1.40 | 1.02 | 508                                                     | 371 | 430 |
| 2-3                        | 440                              | 200 | 280 | 0.96                                                                                          | 1.47 | 1.17 | 422                                                     | 294 | 330 |
| 3-4                        | 394                              | 166 | 343 | 1.00                                                                                          | 1.50 | 1.20 | 394                                                     | 249 | 292 |
| 4-5                        | 261                              | 115 | 244 | 1.12                                                                                          | 1.50 | 1.20 | 292                                                     | 173 | 293 |
| 5-6                        | 220                              | 82  | 235 | 1.12                                                                                          | 1.50 | 1.30 | 246                                                     | 123 | 305 |
| 6-7                        | 150                              | 116 | 233 | 1.10                                                                                          | 1.55 | 1.30 | 165                                                     | 180 | 303 |
| 7-8                        | 145                              | 127 | 190 | 1.10                                                                                          | 1.57 | 1.30 | 160                                                     | 200 | 247 |
| 8-9 (12 P. M.)             | 98                               | 44  | 114 | 1.15                                                                                          | 1.57 | 1.37 | 113                                                     | 69  | 157 |
| 9-10                       | 100                              | 64  | 110 | 1.15                                                                                          | 1.60 | 1.40 | 115                                                     | 102 | 154 |
| 10-10½                     | 17                               | 21  | 58  | 1.16                                                                                          | 1.60 | 1.38 | 20                                                      | 34  | 80  |
| 10½-17¼ <sup>1</sup>       |                                  |     |     |                                                                                               |      |      |                                                         |     |     |
| 17¼-18                     | 10                               | 93  | 45  | 1.20                                                                                          | 1.62 | 1.40 | 12                                                      | 151 | 71  |
| 18-19                      | 0                                | 87  | 85  | 0                                                                                             | 1.62 | 1.40 | 0                                                       | 141 | 119 |
| 19-20                      | 0                                | 95  | 82  | 0                                                                                             | 1.62 | 1.40 | 0                                                       | 154 | 115 |
| 20-21 (12 M.)              | 18                               | 9   | 82  | 1.30                                                                                          | 1.50 | 1.42 | 23                                                      | 13  | 117 |
| 21-22                      | 22                               | 7   | 84  | 1.30                                                                                          | 1.50 | 1.40 | 25                                                      | 11  | 118 |
| 22-23                      | 7                                | 165 | 60  | 1.32                                                                                          | 1.57 | 1.32 | 9                                                       | 260 | 80  |
| 23-24                      | 0                                | 190 | 33  | 0                                                                                             | 1.59 | 1.22 | 0                                                       | 300 | 40  |
| 24-25                      | 3                                | 44  | 21  | 1.30                                                                                          | 1.59 | 1.20 | 4                                                       | 70  | 25  |
| 25-26                      | 40                               | 40  | 20  | 1.38                                                                                          | 1.57 | 1.10 | 55                                                      | 63  | 22  |
| 26-27                      | 45                               | 16  | 32  | 1.38                                                                                          | 1.58 | 1.05 | 62                                                      | 25  | 34  |
| 27-28                      | 110                              | 34  | 35  | 1.38                                                                                          | 1.57 | 0.95 | 152                                                     | 54  | 33  |
| 28-29                      | 115                              | 98  | 78  | 1.35                                                                                          | 1.58 | 0.87 | 155                                                     | 154 | 68  |
| 29-30                      | 138                              | 50  | 129 | 1.33                                                                                          | 1.57 | 0.70 | 183                                                     | 79  | 90  |
| 30-31                      | 161                              | 60  | 45  | 1.28                                                                                          | 1.58 | 0.69 | 205                                                     | 95  | 31  |
| 31-32                      | 120                              | 67  | 56  | 1.28                                                                                          | 1.50 | 0.65 | 153                                                     | 100 | 36  |
| 32-33 (12 P. M.)           | 80                               | 64  | 18  | 1.22                                                                                          | 1.45 | 0.60 | 98                                                      | 93  | 11  |
| 33-34                      | 59                               | 38  | 20  | 1.20                                                                                          | 1.38 | 0.50 | 71                                                      | 52  | 10  |

<sup>1</sup> Record lost for six hours, forty-five minutes.

TABLE IV. — *Continued.*

| Time of suspension in air. | Number of contractions per hour. |      |       | Mean height of contractions during the hour = actual shortening of the muscle in centimetres. |      |      | Work done during one hour expressed as gramcentimetres. |      |      |
|----------------------------|----------------------------------|------|-------|-----------------------------------------------------------------------------------------------|------|------|---------------------------------------------------------|------|------|
| hour                       | a.                               | b.   | c.    | a.                                                                                            | b.   | c.   | a.                                                      | b.   | c.   |
| 34-35                      | 38                               | 33   | 16    | 1.15                                                                                          | 1.30 | 0.45 | 44                                                      | 43   | 7    |
| 35-36                      | 24                               | 29   | 16    | 1.10                                                                                          | 1.25 | 0.40 | 26                                                      | 36   | 6    |
| 36-37                      | 23                               | 19   | 7     | 1.10                                                                                          | 1.20 | 0.04 | 25                                                      | 23   | 3    |
| 37-38                      | 23                               | 58   | 3     | 1.05                                                                                          | 1.20 | 0.03 | 24                                                      | 70   | 1    |
| 38-39                      | 28                               | 32   | 6     | 1.10                                                                                          | 1.22 | 0.02 | 31                                                      | 39   | 1    |
| 39-40                      | 27                               | 33   | 4     | 1.10                                                                                          | 1.22 | 0.02 | 30                                                      | 40   | 1    |
| 40-41                      | 32                               | 46   | 7     | 1.11                                                                                          | 1.22 | 0.01 | 36                                                      | 56   | 1    |
| 41-42                      | 60                               | 48   | 4     | 1.05                                                                                          | 1.20 | 0.01 | 63                                                      | 58   | 0    |
| 42-43                      | 57                               | 60   | 14    | 1.00                                                                                          | 1.05 | 0.01 | 57                                                      | 63   | 2    |
| 43-44                      | 69                               | 60   | 13    | 1.00                                                                                          | 1.08 | 0.01 | 69                                                      | 64   | 1    |
| 44-45 (12 M.)              | 88                               | 34   | 19    | 0.98                                                                                          | 1.05 | 0.02 | 86                                                      | 36   | 4    |
| 45-46                      | 80                               | 37   | 28    | 0.90                                                                                          | 1.05 | 0.01 | 72                                                      | 39   | 3    |
| 46-47                      | 83                               | 37   | 23    | 0.80                                                                                          | 1.05 | 0    | 66                                                      | 39   | 0    |
| 47-48                      | 112                              | 37   | 50    | 0.70                                                                                          | 1.02 | 0    | 78                                                      | 38   | 0    |
| 48-49                      | 154                              | 42   | 75    | 0.55                                                                                          | 1.00 | 0    | 85                                                      | 42   | 0    |
| 49-50                      | 78                               | 42   | trace | 0.25                                                                                          | 0.98 | 0    | 20                                                      | 41   | 0    |
| 50-51                      | 10                               | 42   |       | 0.05                                                                                          | 0.82 |      | 0                                                       | 35   |      |
| 51-52                      |                                  | 16   |       |                                                                                               | 0.75 |      |                                                         | 12   |      |
| 52-53                      |                                  | 37   |       |                                                                                               | 0.70 |      |                                                         | 12   |      |
| 53-54                      |                                  | 40   |       |                                                                                               | 0.60 |      |                                                         | 26   |      |
| 54-55                      |                                  | 44   |       |                                                                                               | 0.60 |      |                                                         | 24   |      |
| 55-56                      |                                  | 55   |       |                                                                                               | 0.62 |      |                                                         | 34   |      |
| 56-57 (12 P. M.)           |                                  | 48   |       |                                                                                               | 0.60 |      |                                                         | 29   |      |
| 57-58                      |                                  | 42   |       |                                                                                               | 0.52 |      |                                                         | 22   |      |
| 58-59                      |                                  | 45   |       |                                                                                               | 0.45 |      |                                                         | 20   |      |
| 59-60                      |                                  | 34   |       |                                                                                               | 0.22 |      |                                                         | 8    |      |
| 60-61                      |                                  | 34   |       |                                                                                               | 0.07 |      |                                                         | 2    |      |
| 61-62                      |                                  | 16   |       |                                                                                               | 0.05 |      |                                                         | 1    |      |
| Total                      | 4817                             | 3762 | 3877  |                                                                                               |      |      | 4915                                                    | 4958 | 4061 |

The strips of muscle were weighed as carefully as possible before suspending them, and also at the close of the treatment with inorganic salts. At each weighing the moisture was removed as nearly



as possible to the same extent by draining on glazed porcelain or on glass. The strips were then weighed between watch crystals. The results of all experiments where double weighings were made show a loss of weight. This fact is of significance, although I have not made a sufficient number of experiments for quantitative estimates from this standpoint.

TABLE V.

Weight of muscle strips at the beginning and end of treatment with Ringer's solution.

| Number of the experiment. | Weight before treatment with Ringer's solution. | Weight after treatment with Ringer's solution. | Time of suspension in Ringer's solution. | Loss.       |
|---------------------------|-------------------------------------------------|------------------------------------------------|------------------------------------------|-------------|
| 42 <i>a</i>               | 0.321 grams                                     | 0.196 grams                                    | 51½ hours                                | 0.125 grams |
| 42 <i>b</i>               | 0.283 "                                         | 0.231 "                                        | 72 "                                     | 0.052 "     |
| 42 <i>c</i>               | 0.340 "                                         | 0.232 "                                        | 50 "                                     | 0.108 "     |

The figures in this table, which express the weights before the use of Ringer's solution, are of the greatest importance for the present consideration. The three strips used gave respectively 4915, 4958, and 4061 gramcentimetres of recorded work. If this work be converted into its heat equivalent it may then be compared with the heat equivalents of the possible sources of energy-giving material. One gramcentimetre of work equals 980 ergs; one calorie equals  $4.2 \times 10^7$  ergs; one gramcentimetre therefore equals  $0.00002\frac{1}{3}$  calories.

The total heat of oxidation of one gram of proteid and of one gram of cane-sugar varies according to the determinations of different investigators. Stohman gives for beef (fat free) 5641, for urea 2465, and for cane-sugar 3959 calories respectively. Danilewsky gives for fibrin 5772, urea 2537, and for cane-sugar 4176 calories. Rubner gives for muscle extracted with water 5778, and for urea 2523 calories. If the oxidation equivalent of the urea formed as a result of the metabolism of one gram of proteid, *i.e.*, one third of a gram of urea, is deducted, it may be assumed that, in round numbers, the average oxidation energy of one gram of proteid or one gram of sugar available for muscular metabolism is 4890 calories for proteid, or 4000 calories for carbohydrate.

The amount of work recorded by the three strips is the equivalent of the oxidation of 0.0000233, 0.0000235, and 0.0000193 grams of

proteid or of 0.0000287, 0.0000289, 0.0000234 grams of carbohydrate respectively. These amounts represent the oxidation equivalents if all the energy appears as work, whereas it is well known that only a small fraction of the total energy of metabolism can be utilized as work. Gaule,<sup>7</sup> 1878, computed that, at least, not less than eight per cent of the energy of contraction in the frog's heart may be recorded as work. The well-known experiments of Fick upon striated muscles show that under the most favorable conditions twenty per cent may be regarded as a maximum yield in work. In the cardiac strip, it must be remembered that many of the fibres are cut across so that their contractions are lost, while others may by their contractions oppose those which exert a direct pull on the lever. It is, therefore, a liberal estimate to assume that fifteen per cent of the energy of oxidation may take the form of work during contractions of the ventricular strip, and that not more than two-thirds of this,—ten per cent of the total energy, is recovered on the record. These facts are arranged for comparison in the following table:—

TABLE VI.

| Number of the experiment. | Work recorded. | Mechanical equivalent in heat. | Total oxidation equivalent in proteid. | Equivalent in proteid of metabolism if 10% is recovered as work. |
|---------------------------|----------------|--------------------------------|----------------------------------------|------------------------------------------------------------------|
| 42 <i>a</i>               | 4915 gr. cm.   | 0.11468 calories               | 0 0000233 grams                        | 0.000233 grams                                                   |
| 42 <i>b</i>               | 4958 “         | 0.11569 “                      | 0.0000235 “                            | 0.000235 “                                                       |
| 42 <i>c</i>               | 4061 “         | 0.09312 “                      | 0.0000193 “                            | 0.000193 “                                                       |

The average amount of serum-albumin in the serum of this species of terrapin during the winter season, as determined by Howell<sup>21</sup> in 1884, is 0.69 per cent. If the energy of the heart muscle is supplied by the metabolism of serum-albumin, as Kronecker holds, the amount of blood required to supply the necessary albumin for each of the three strips mentioned above must have been, respectively, 11.5, 13.1, and 9 per cent of their initial weights. But the percentage of blood in the entire body (determined upon mammals) is only 7.7 per cent, a large proportion of which is contained in the great vessels. The percentages exhibited in the following table reduce the question to an absurdity in so far as considering serum-albumin

as the source of the motor energy of the washed strips of the terrapin's heart is concerned.

TABLE VII.

| Number of the experiment. | Work recorded. | Equivalent in the metabolism of serum-albumin of | Weight of serum necessary to supply the serum-albumin. | Initial weight of the muscles. | Proportion of serum in the muscles necessary to account for the work, etc. |
|---------------------------|----------------|--------------------------------------------------|--------------------------------------------------------|--------------------------------|----------------------------------------------------------------------------|
| 42 <i>a</i>               | 4915 gr. cms.  | 0.000233 grams                                   | 0.0338 grams                                           | 0.321 grams                    | 10.5%                                                                      |
| 42 <i>b</i>               | 4958 "         | 0.000235 "                                       | 0.0340 "                                               | 0.283 "                        | 12.0%                                                                      |
| 42 <i>c</i>               | 4061 "         | 0.000193 "                                       | 0.0280 "                                               | 0.340 "                        | 8.2%                                                                       |

The amount of paraglobulin in the blood of this terrapin is, according to Howell, comparatively large, 4.66 grams per 100 c. c. The equivalent of the above work may be expressed in terms of paraglobulin (Table VIII.).

TABLE VIII.

| Number of the experiment. | Work recorded. | Equivalent in the metabolism of paraglobulin. | Weight of serum necessary to supply the paraglobulin. | Initial weight of the muscles. | Proportion of serum in the muscle strips necessary to supply the paraglobulin. |
|---------------------------|----------------|-----------------------------------------------|-------------------------------------------------------|--------------------------------|--------------------------------------------------------------------------------|
| 42 <i>a</i>               | 4915 gr. cms.  | 0.000233 grams                                | 0.0050 grams                                          | 0.321 grams                    | 1.6%                                                                           |
| 42 <i>b</i>               | 4958 "         | 0.000235 "                                    | 0.0050 "                                              | 0.283 "                        | 1.8%                                                                           |
| 42 <i>c</i>               | 4061 "         | 0.000193 "                                    | 0.0041 "                                              | 0.340 "                        | 1.2%                                                                           |

When it is remembered that the heart was irrigated with 0.7 per cent sodium chloride solution for one hour, and that the washing was facilitated by the contractions of the ventricle and by massage during this time, then the possibility of there being even this percentage of blood left in the capillaries or in the meshes of the tissues seems improbable.

The percentage of sugar in systemic blood varies from 0.1 per cent to 0.15 per cent. On this basis it would be utterly impossible to

account for the work, assuming that the energy came from the consumption of sugar furnished by the serum still left in the strips.

The more rational view applicable to this case is that the heart has stored material in its cells, and that the contractions of the isolated ventricular strips are at the expense of this material. The voluntary muscles are admittedly able to contract at the expense of stored contractile material. Why, therefore, should this function be denied to cardiac muscle?

The above experiment seems to demonstrate, beyond doubt, that *the terrapin's heart contracts at the expense of an antecedent contractile substance stored up in its own tissue.*

The amount of work developed by the heart strips under the influence of sodium chloride solution, or, in fact, any of the inorganic solutions used in these experiments, is perfectly represented by the comparison of the height and rate of contractions as discussed throughout this paper under the various headings, since the load was kept uniformly at one gram with all the levers used in the experiments quoted.

#### EXHAUSTION OF HEART MUSCLE.

The word "exhaustion" as applied to the heart has been used to express many different states. At the present time it can no longer be employed without specifying the condition to which it applies. Before defining its use in this section, it will be interesting to sketch, briefly, its use in the literature on the subject.

In 1874 Kronecker and Stirling<sup>8</sup> found that a frog's heart filled with 0.6 per cent sodium chloride solution soon ceased to beat, and could be made to beat again only on the introduction of pure or diluted serum. They considered this condition brought on by sodium chloride solution one of exhaustion. From their experiments they were led to the conclusion that the heart in its contractions used material obtained directly from the blood. According to the view proposed by Kronecker and Stirling at that time, the frog's heart is exhausted in sodium chloride solution because the material of the blood necessary to each contraction is washed out.

Investigations tending to support and develop Kronecker's views were made by Stiénon,<sup>6</sup> 1878, Martius,<sup>9</sup> 1882, Kronecker and Popoff,<sup>12</sup> 1887, and White,<sup>17</sup> 1896.

In 1878 Gaule<sup>7</sup> showed that a heart which is quiet in sodium chloride could be made to contract for several hours by using a solu-



tion of alkali-saline, and that in this latter solution it gave off as many as one thousand contractions. This remarkable result can be explained, he says, only on the supposition that the muscle of the heart has the antecedent material which it uses in contractions stored up in its substance. When a heart will no longer beat in renewed alkaline sodium chloride solution, according to Gaule, it can be revived only by feeding it with blood. Apparently, according to his view, a heart is exhausted when its store of contractile material is used up.

In 1882 Martius,<sup>9</sup> working under the direction of Kronecker, ascribed the beneficial effect of alkali-saline not to a more complete utilization of stored material in the heart cells, as Gaule supposed, but to the fact that the alkali, by combining with carbon dioxide, prevented asphyxiation of the contractile tissue, and thus permitted a more complete utilization of the remnants of blood still in the interstices of the heart. Only when this material is used up is a heart truly exhausted. Martius stated that such a heart could be revived only by the use of substances like blood, serum, lymph, *i.e.*, substances containing serum-albumin, and he concluded that serum-albumin was the particular element in the blood used by the cardiac muscle in contraction.

In 1883 and 1885 Ringer<sup>10</sup> gave a new interpretation to the term "exhaustion," when he said "calcium salts are necessary for the proper contractions of the heart, yet they must be antagonized by potassium salts." According to this idea, a heart fails to contract in sodium chloride or in alkali-saline, because it is not supplied with the necessary calcium and potassium salts. Ringer's standpoint was still further emphasized by Howell and Cooke<sup>15</sup> in 1893, who showed that hearts that had ceased to beat after abundant irrigation with saline or alkali-saline could be revived and kept in normal contractions for long periods when supplied with Ringer's solution or with extracts of milk, blood, or gastric juice that contained only traces of proteid.

When in 1896 White<sup>17</sup> wrote in support of Kronecker's view he was compelled to take refuge in an hypothesis which attempts to overthrow the most painstaking work by one word. This hypothesis assumes that the heart may beat on infinitesimal, and, one may add, undetectable quantities of serum-albumin, and that it is almost if not quite impossible to completely remove all traces of serum-albumin from the muscular spaces of the heart.

White said that by the methods used by Merunowicz, Aubert, Martius, and other investigators, "it was impossible to completely wash out a heart." He held, therefore, that their conclusions were unfounded. He said that a heart must be irrigated through the most improved perfusion cannula, that it must be irrigated by successive solutions of sodium chloride, alkali-sodium chloride, and Ringer's solution until it is quiet. Only when it no longer responds to any of these solutions is it free from serum-albumin and truly exhausted. This is, however, the familiar argument in a circle, since the conclusion proceeds directly from the hypothesis assumed in the beginning. White also committed the grave error of not using the most favorable combination of salts in Ringer's solution, and, therefore, did not secure completely washed-out hearts in the sense in which he uses the term. By his own process of reasoning his results must be placed in the same category as those of the earlier investigators. In four examples that he gives, the hearts were exhausted after a total time of irrigation of: 1 hour, 25 minutes, 5 hours, 30 minutes, 9 hours, 15 minutes, and 4 hours, 45 minutes.

In this laboratory, in recent experiments upon the whole heart of the frog, the results of which will be published later, it has been observed that after the heart has ceased to beat upon the Ringer's mixture used by White it may still beat, and beat well, upon the mixture generally employed in this paper (0.7% NaCl, 0.026%  $\text{CaCl}_2$ , and 0.03% KCl). Moreover, with many hearts, although not with all, it has happened that after they have ceased to beat upon this last mixture the gradual increase of the amount of calcium salts in the Ringer's solution called forth new beats for a considerable period, an effect also obtained on heart strips, as previously described in this paper.

From the above review it will be seen that the term "exhaustion," as applied to the heart, either expresses states in which the inorganic salts necessary to the contractions of the heart are removed or disturbed in their relations, or it expresses states in which the antecedent organic contractile material is consumed or removed. In the first group may be included as many conditions of exhaustion as there are combinations of the inorganic salts that will not support contractions. That the series of beats obtained with any solution should disappear by successively smaller contractions, as they do in a heart filled with sodium chloride solution, is non-essential. In the second group must be included, on the one

hand, Kronecker's view, that exhaustion signifies a lack of sufficient free serum-albumin surrounding the muscle tissue to support contractions, and, on the other, Gaule's view, that exhaustion means a lack of antecedent contractile material stored in the cardiac tissue.

It seems to me it would be better to restrict the application of the term "exhaustion" to states of the cardiac tissue itself, and to designate in some other way all those conditions which imply the presence or absence of some substance or substances in the surrounding blood or artificial fluid. My own experiments are full of examples that demonstrate the inefficiency of sodium chloride solution to produce a true exhaustion in the ventricular strip. Time after time the sodium chloride solution series has been almost exactly duplicated on the same strip after an intervening recovery, due to a bath of solutions of inorganic salts alone. It is admitted that an inorganic diet cannot serve directly as a source of energy. My experiments give no convincing proof of the ability of the isolated muscle to use even the organic material of serum. In Experiment 42, given above, strips *a*, *b*, and *c* were made to contract incompletely with serum after they could no longer be aroused by Ringer's solution; but *b* gave only the slightest movements when treated with serum, and in both *a* and *c* the revival of contractions due to serum treatment did not last as long as did the contractions of *b*, still moist with the inorganic salt solution. It is possible, therefore, that the serum effect on *a* and *c* was due entirely to the more favorable relation of the inorganic salts, and produced only a more perfect exhaustion of the contractile material stored in the muscle cells of the strips.

The particular type of dying out of the contractions given by the strips in Experiment 42, quoted above, in which, after many hours, the amplitude of contractions slowly but steadily decreases to a zero from which no good recovery can be obtained, has occurred so often that it may be expected with confidence whenever muscular strips of the terrapin's ventricle are treated with any solution favorable to the development of continuous rhythmic contractions. In fact, in the very first experiment of this investigation, a heart strip, after seventy-two hours' continuous contraction, ceased in the same way as strips *a* and *b* of the above experiment. The strip of this first experiment was suspended in a muscle moist-chamber, and was made to begin its series of contractions by moistening it with serum much diluted with 0.6 per cent sodium chloride.

This type of dying out of the contractions I take to indicate a using up of the organic contractile material in the muscle. The completeness of exhaustion in this sense depends upon whether the muscular strip is subjected to the most favorable relation of the inorganic salts of the blood, especially of the sodium, potassium, and calcium salts. Whether or not the isolated strip is capable of being nourished, that is, of utilizing the stored energy of the organic constituents of the liquid in which it is immersed, is an independent question.

#### SUMMARY.

1. A bath of normal serum will not keep a strip from the apex of terrapin ventricle in contraction, although it keeps it in good condition for contraction for three or four days. By slightly increasing the amount of calcium chloride in the serum regular contractions may be produced.

2. An artificial mixture of sodium, potassium, and calcium salts in the proportions in which they exist in serum acts like serum, in that it does not produce a continuous series of rhythmic contractions, but sustains the cardiac strip in good condition for contraction, for at least three days. For the terrapin's heart this proportion is approximately 0.7 per cent sodium chloride, 0.026 per cent calcium chloride, and 0.03 per cent potassium chloride.

3. Sodium chloride will produce and sustain contractions for a short time only, and the series of contractions presents the appearance of fatigue. This appearance of fatigue indicates only the removal of the inorganic salts necessary to contraction, and is not an exhaustion of the contractile substance of the muscle.

4. Calcium salts in isotonic solutions of sodium chloride stimulate the cardiac strip to increased rhythm and final permanent contracture.

5. Potassium chloride in isotonic solutions of sodium chloride prevents contractions, and keeps the ventricular strip in a state of relaxation.

6. There is an optimum ratio of the potash, calcium, and sodium salts in isotonic solution most favorable to the development and maintenance of the contractions in the ventricular strip. For the apex of the ventricle of the terrapin this proportion is sodium chloride 0.7 per cent, calcium chloride 0.04 or 0.05 per cent, potassium chloride 0.03 per cent, if the strip is fresh and filled with blood.



If the strip is from a heart that has been washed with 0.7 per cent sodium chloride, the proportion of salts given in conclusion 2, above, is the most favorable to the maintenance of contractions. The rhythm in the spongy ventricular strip is rarely perfectly regular in this solution.

7. Complete exhaustion of the contractile substance in the heart of the winter terrapin is brought about by the use of inorganic salt solution only after thirty to seventy-two hours' continuous rhythmic activity, or by seventy-two to one hundred hours' suspension, if the activity has been slight.

8. Cane sugar and urea in isotonic solutions do not produce rhythmic contractions in the isolated strip. Dextrose in isotonic solution throws the strip into strong tone and may produce an imperfect series of contractions.

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## BIOGRAPHY.

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